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TITLE: HUMAN PAPILLOMA VIRUS TREATMENT

APPLICANT: JOHN R. NEEFE, STEPHEN E. GOLDSTONE, MARK T. WINNETT AND MARVIN SIEGEL

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HUMAN PAPILLOMA VIRUS TREATMENT

Cross Reference to Related Applications

This application claims priority from U.S. Provisional Application No. 60/214,202, filed June 26, 2000, the content of which is incorporated herein by reference in its entirety.

Field of the Invention

The invention relates to therapies for human papilloma virus infections.

Background of the Invention

Infection with human papilloma virus (HPV) is common. HPV can be transmitted sexually, and it is estimated that 20-80% of sexually active adults have been infected. While a majority of infections are asymptomatic, infection can lead to the development of genital warts (which have a prevalence of about 1-5% among adults) and cancer of the anogenital tract. Another type of cancer, cervical cancer, is strongly associated with HPV (Frazer, *Genitourin. Med.* 72:398-403, 1996). HPV types 6, 11, 16, 18, 31, and 33 are often associated with an increased risk of cancer, with types 16 and/or 18 being detected in more than 90% of cervical carcinomas (van Driel *et al.*, *Ann. Med.* 28:471-477, 1996). Types 6 and 11 are also associated with anogenital warts. For reviews of papilloma viruses and their associated pathologies, see Shah *et al.*, "Chapter 66: Papillomaviruses," In: *Virology*, 3rd Edition, Fields *et al.*, Eds., Raven Press, Philadelphia, pp 2077-2109, 1996, and zur Hausen, *J. Natl. Cancer Inst.* 92:690-698, 2000.

There is currently no safe and effective way to treat or prevent warts or the diseases described above by targeting the immune system. Efforts to develop such therapies have been hampered for several reasons, one of which is the dogma that antigens from a single HPV type elicit a limited, type-specific immune response. Consequently, it has been suggested that a cocktail containing antigens from several different HPV types is necessary for a broadly effective HPV therapy (Caine *et al.*, *Science* 288:1753, 2000).

Summary of the Invention

The present invention is based, in part, on the discovery that a fusion protein containing a protein from one HPV type can be used to treat a disease or condition that is caused by infection with another HPV type. For example, an HPV type 16 antigen, fused to a bacterial heat shock protein (hsp), was effective in treating human anogenital warts caused by HPV types other than type 16 (*e.g.*, HPV types 6 and 11). This result supports two contentions: (1) that warts can be treated with an HPV protein and (2) that therapeutic agents aimed at HPV need not contain protein antigens from different HPV types in order to be broadly effective.

Accordingly, the invention features a method of treating a wart in a subject by administering to the subject a composition containing (1) an hsp, or an immunostimulatory fragment thereof, and (2) an HPV protein (*e.g.*, an antigenic protein such as the E7 protein of, *e.g.*, HPV type 16) or an antigenic fragment thereof. These components may be referred to herein as "component (1)" and "component (2)," respectively. The hsp (or the immunostimulatory fragment thereof) and the HPV protein (or the antigenic fragment thereof) can be either simply combined in the same preparation or more closely associated by chemical conjugation or fusion (*i.e.*, one can administer a fusion protein having the components described herein or a nucleic acid molecule that encodes it). When combined, conjugated, or fused, component (1) and component (2) would be administered simultaneously. Each component can, however, also be administered separately (*e.g.*, sequentially), and component (2) can be administered without component (1). The method described above can include a step in which a subject who has, or who is suspected of having, a wart is identified (in the context of treating the subject, identification would be made before administration of the therapeutic agent begins). Physicians and others of ordinary skill in the art are well able to identify such subjects.

The methods of the invention can also be used to prevent a wart, in which case a subject who desires, or who would benefit from, wart prevention (rather than a subject who already has a wart) is identified.

The invention also features methods of treating a subject who has a disease or condition caused by an infection with an HPV of a first type (*e.g*, type 5, 6, 11, 18, 31, 33, 35, 45, 54, 60, or 70) by administering to the subject a composition containing (1) an hsp, or

an immunostimulatory fragment thereof, and (2) a protein of an HPV of a second type (*e.g.*, type 16) or an antigenic fragment thereof. That is, the HPV of the "first type" and the HPV of the "second type" are different from one another; they are of two different HPV types. The hsp (or the immunostimulatory fragment thereof) and the HPV protein (or the antigenic fragment thereof) can be either simply combined in the same preparation or more intimately associated by chemical conjugation or fusion (*i.e.*, one can administer a fusion protein having the components described herein or a nucleic acid molecule that encodes it). When combined, conjugated, or fused, component (1) and component (2) would be administered simultaneously. Each component can, however, also be administered separately (*e.g.*, sequentially), and component (2) can be administered without component (1). Here again, the method can include a step in which a subject who has, or is suspected of having, an HPV infection (or a disease or condition associated therewith) is identified.

When a subject who is infected with a first HPV type is given a composition that includes an HPV of a second type, the method can be carried out before an HPV infection is typed, before it is manifest, or before it has occurred (*i.e.*, one need not know the particular HPV type a subject has been infected with, or will be infected with, before treatment or prophylaxis can begin). When the methods are preventative, they can include a step in which a subject who desires, or who would benefit from, prevention of an HPV infection is identified.

The compositions described herein can be administered in amounts that are sufficient to treat the wart (by, for example, reducing the size or altering the shape of the wart, or by ameliorating a symptom associated with a wart (*e.g.*, the pain often associated with a plantar wart); when a subject has more than one wart, treatment can encompass reducing the number of warts). Similarly, the compositions described herein can be administered in amounts that are sufficient to treat the disease (*e.g.*, cancer (such as cervical cancer or anal cancer) or other condition (*e.g.*, dysplasia (such as cervical or anal dysplasia)) that is caused by, or associated with, an HPV infection. Although warts are mentioned separately above, warts also constitute a condition caused by or associated with HPV. Physicians and others of ordinary skill in the art will recognize an effective "treatment" of a wart or an HPV-associated disease or condition when there is a diminution in an undesirable physiological affect associated with the wart or the disease or condition. The clinical and physiological manifestations of a wart,

as well as those of a disease or condition associated with HPV infection, are discussed in, for example, Fauci *et al.*, *Harrison's Principles of Internal Medicine*, 14th Edition, McGraw-Hill Press, New York, pp 302-303 and 1098-1100, 1998.

"Subjects" who can benefit from the methods described herein are those who can be infected by papilloma viruses (*e.g.*, mammals such as humans, livestock (*e.g.*, cows, horses, pigs, sheep, and goats), and domestic animals (*e.g.* cats and dogs)). The wart can be one that occurs on the subject's genitalia, skin, or internal organs (such as the warts that appear on the vocal cords in recurrent respiratory papillomatosis (RRP; also known as juvenile laryngeal papillomatosis (JLP) or adult-onset RRP)).

The invention further includes the use of one or more of the compositions described herein (including those that contain proteins, protein conjugates or fusion proteins, or the nucleic acid molecules that encode them) for the treatment of subject who has warts or a disease or conditions associated with (or caused by) an HPV infection, in accordance with the methods described herein. The invention further includes the use of one or more of such compositions in the manufacture of a medicament for the treatment of subject who has warts or a disease or conditions associated with (or caused by) an HPV infection, in accordance with the methods described herein.

An "antigenic fragment" of a protein (*e.g.* an HPV protein) is any portion of the protein that, when administered in accordance with the methods described herein, elicits, in a subject, an immune response that is either a fragment-specific or specific for the protein from which the fragment was obtained. The immune response can be either a humoral or a cell-mediated response. For example, an antigenic fragment can be an HLA class I peptide antigen, such as described below. One of ordinary skill in the art will recognize that the immune response desired in the context of the present invention can be generated not only by intact proteins and fragments thereof, but also by mutant proteins (*e.g.*, those that contain one or more additions, substitutions (*e.g.* conservative amino acid substitutions) or deletions in their amino acid sequence). Mutant HPV antigens can be readily made and tested for their ability to work in the context of the present invention.

An "immunostimulatory fragment" of a protein (*e.g.*, an hsp) is any portion of the protein that, when administered in accordance with the methods described herein, facilitates an immune response by an antigen. For example, if the immune response to an HPV protein

is facilitated when that HPV protein is administered with (*e.g.*, fused to) a fragment of an hsp, that fragment is an immunostimulatory fragment of an hsp. One of ordinary skill in the art will recognize that the immune response can also be facilitated by mutant hsps (*e.g.*, hsps that contain one or more additions, substitutions (*e.g.*, conservative amino acid substitutions) or deletions in the amino acid sequence). Mutant hsps can be readily made and tested for their ability to facilitate an immune response to an HPV antigen.

The methods of the invention provide an efficient means of: (1) treating or preventing warts and (2) treating or preventing a disease or condition caused by (or associated with) an infection with one HPV type with (*i.e.*, using) a composition containing an HPV of another type. Consequently, a composition containing an HPV antigen of a single HPV type can be used in many, if not most, subjects, regardless of the HPV type with which they are infected (or with which they may become infected). It is surprising that HPV compositions are effective in these circumstances (*i.e.*, circumstances requiring cross-reactivity). It has been thought that HPV antigens of one type cannot elicit an effective immune response against another type. Other features or advantages of the present invention will be apparent from the following detailed description, and also from the claims.

Detailed Description

The invention relates to broadly effective HPV-based therapeutic agents containing an hsp and an HPV protein (*e.g.*, a protein antigen). Without limiting the invention to methods in which HPV-based therapeutics exert their effect through a particular mechanism, the agents are thought to produce an immune response that improves warts and other conditions (*e.g.*, dysplasia) and diseases (*e.g.*, cancer) associated with HPV infections. Notably, while the compositions of the invention may contain an HPV protein from more than one HPV type, they can contain an HPV protein from only a single type. Moreover, compositions that contain an HPV protein from a single HPV type are useful in treating or preventing warts or other HPV-associated diseases or conditions that are caused by an HPV infection of another (*i.e.*, a different) type. Various materials and procedures suitable for use in connection with the invention are discussed below.

Preparation of Fusion Proteins

The nucleic acid sequences encoding hsps and HPV proteins are known and available to those of ordinary skill in the art. Thus, nucleic acid constructs encoding fusion polypeptides useful in the methods of the invention can be readily prepared using routine methods (similarly, such nucleic acid molecules can be used to produce hsps and HPV proteins individually; the individual hsps and HPV proteins can then be physically combined (*e.g.* simply mixed together) or joined by chemical conjugation (see below) or via disulfide bonds). Examples of nucleic acid sequences that encode an hsp optionally fused to an antigen (*e.g.*, an HPV antigen) can be found in International Publication Nos. WO 89/12455, WO 94/29459, WO 98/23735, and WO 99/07860 and the references cited therein. Methods by which proteins, including fusion proteins, can be expressed and purified are discussed further below.

Preparation of Protein Conjugates

Component (1) and component (2) can also be joined by post-translational conjugation of individual hsps and individual HPV antigens. Methods for chemically conjugating two proteins (or portions thereof) are known in the art (*see, e.g.*, the techniques described in Hermanson, *Bioconjugate Techniques*, Academic Press, San Diego, CA, 1996; Lussow *et al.*, *Eur. J. Immun.* 21:2297-2302, 1991; and Barrios *et al.*, *Eur. J. Immun.* 22:1365-1372, 1992). Conjugates can be prepared by methods that employ cross-linking agents, such as glutaraldehyde (which becomes a part of the resultant conjugate), or that join component (1) and component (2) by disulfide bonds. One can use cysteine residues that are either naturally present or recombinantly inserted in the hsp, the HPV antigen, or both, to facilitate intermolecular disulfide bond formation. Compositions containing hsps or immunostimulatory fragments thereof (*i.e.* component (1)) that are non-covalently associated with HPV antigens can be produced as described in U.S. Patent Nos. 6,048,530; 6,017,544; 6,017,540; 6,007,821; 5,985,270; 5,948,646; 5,935,576; 5,837,251; 5,830,464; or 5,750,119. See also, U.S. Patent Nos. 5,997,873; 5,961,979; 6,030,618; 6,139,841; 6,156,302; 6,168,793; and International Publication No. WO 97/06821.

Regardless of the final configuration of the composition administered, component (1) and component (2) can include the following.

HPV protein antigens

Any HPV antigen is suitable for use in the compositions (*e.g.*, the mixtures, conjugates and fusion proteins described herein) of the present invention. However, HPV antigens that express recognizable epitopes on the surface of an HPV infected cell should be especially useful. HPV expresses six or seven non-structural proteins and two structural proteins, and each of these can serve as a target in the immunoprophylactic or immunotherapeutic approaches described herein.

The viral capsid proteins L1 and L2 are the late structural proteins. L1 is the major capsid protein, the amino acid sequence of which is highly conserved among different HPV types. There are seven early non-structural proteins. Proteins E1, E2, and E4 play an important role in virus replication. Protein E4 also plays a role in virus maturation. The role of E5 is less well known. Proteins E6 and E7 are oncoproteins that are critical for viral replication, as well as for host cell immortalization and transformation.

Hsps

A variety of hsps have been isolated, cloned, and characterized from a diverse array of organisms (Mizzen, *Biotherapy* 10:173-189, 1998; as used herein, the term "heat shock protein(s)" or its abbreviation (hsp(s)) is synonymous with, or encompasses, the proteins referred to as "stress proteins"). Immunostimulatory hsps, or immunostimulatory fragments thereof, are suitable for use in the compositions described herein (*e.g.*, as part of a fusion polypeptide). Hsp70, hsp60, hsp20-30, and hsp10 are among the major determinants recognized by host immune responses to infection by *Mycobacterium tuberculosis* and *Mycobacterium leprae*. In addition, hsp65 of Bacille Calmette Guerin (BCG), a strain of *Mycobacterium bovis*, was found to be an effective immunostimulatory agent, as described in the example below.

Families of hsp genes and hsps, any of which can be used as described herein for component (1), are well known in the art. These include, for example, Hsp100-200, Hsp100, Hsp90, Lon, Hsp70, Hsp60, TF55, Hsp40, FKBPs, cyclophilins, Hsp20-30, ClpP, GrpE, Hsp10, ubiquitin, calnexin, and protein disulfide isomerases. *See, e.g.*, Macario, *Cold Spring Harbor Laboratory Res.* 25:59-70, 1995; Parsell *et al.*, *Rev. Genet.* 27:437-496, 1993; and

U.S. Patent No. 5,232,833. The hsp can be, but is not limited to, a mammalian, bacterial, or mycobacterial hsp.

Grp170 (for glucose-regulated protein) is an example of an hsp in the hsp100-200 family. Grp170 resides in the lumen of the endoplasmic reticulum, in the pre-Golgi compartment, and may play a role in immunoglobulin folding and assembly.

Examples of hsps in the hsp100 family include mammalian Hsp110, yeast Hsp104, and the *E. coli* hsps ClpA, ClpB, ClpC, ClpX and ClpY.

Examples of hsps in the hsp90 family includes HtpG in *E. coli*, Hsp83 and Hsc83 in yeast, and Hsp90alpha, Hsp90beta, and Grp94 in humans. Hsp90 binds groups of proteins that are typically cellular regulatory molecules, such as steroid hormone receptors (*e.g.*, glucocorticoid, estrogen, progesterone, and testosterone receptors), transcription factors, and protein kinases that play a role in signal transduction mechanisms. Hsp90 proteins also participate in the formation of large, abundant protein complexes that include other stress proteins.

Lon is a tetrameric ATP-dependent protease that degrades non-native proteins in *E. coli*.

Examples of hsps in the hsp70 family include Hsp72 and Hsc73 from mammalian cells, DnaK from bacteria or mycobacteria such as *Mycobacterium leprae*, *Mycobacterium tuberculosis*, and *Mycobacterium bovis* (such as Bacille-Calmette Guerin; referred to herein as hsp71), DnaK from *E. coli*, yeast, and other prokaryotes, and BiP and Grp78. Hsp70 is capable of specifically binding ATP as well as unfolded polypeptides and peptides; hsp70 participates in protein folding and unfolding as well as in the assembly and disassembly of protein complexes.

An example of an hsp from the Hsp60 family is Hsp65 from mycobacteria. Bacterial Hsp60 is also commonly known as GroEL. Hsp60 forms large homooligomeric complexes, and appears to play a key role in protein folding. Hsp60 homologues are present in eukaryotic mitochondria and chloroplasts.

Examples of hsps in the TF55 family include Tcpl, TRiC, and thermosome. These proteins typically occur in the cytoplasm of eukaryotes and some archaebacteria, and they form multi-membered rings, promoting protein folding. They are also weakly homologous to Hsp60.

Examples of hsps in the Hsp40 family include DnaJ from prokaryotes such as *E. coli* and mycobacteria and HSJ1, HDJ1, and Hsp40. Hsp40 plays a role as a molecular chaperone in protein folding, thermotolerance and DNA replication, among other cellular activities.

Examples of FKBPs include FKBP12, FKBP13, FKBP25, and FKBP59, Fprl and Nepl. These proteins typically have peptidyl-prolyl isomerase activity and interact with immunosuppressants such as FK506 and rapamycin. The proteins are typically found in the cytoplasm and the endoplasmic reticulum.

Examples of cyclophilins include cyclophilins A, B, and C. These proteins have peptidyl-prolyl isomerase activity and interact with the immunosuppressant cyclosporin A.

Hsp20-30 is also referred to as small Hsp. Hsp20-30 is typically found in large homooligomeric complexes or possibly heterooligomeric complexes. An organism or cell type can express several different types of small Hsps. Hsp20-30 interacts with cytoskeletal structures and may play a regulatory role in the polymerization/depolymerization of actin. Hsp20-30 is rapidly phosphorylated upon stress or exposure of resting cells to growth factors. Hsp20-30 homologues include alpha-crystallin.

ClpP is an *E. coli* protease involved in degradation of abnormal proteins. Homologues of ClpP are found in chloroplasts. ClpP forms a heterooligomeric complex with ClpA.

GrpE is an *E. coli* protein of about 20 kDa that is involved in the rescue of stress-damaged proteins as well as the degradation of damaged proteins. GrpE plays a role in the regulation of stress gene expression in *E. coli*.

Hsp10 examples include GroES and Cpn10. Hsp10 is found in *E. coli* and in the mitochondria and chloroplasts of eukaryotic cells. Hsp10 forms a seven-membered ring that associates with Hsp60 oligomers. Hsp10 is also involved in protein folding.

Ubiquitin has been found to bind proteins in coordination with the proteolytic removal of the proteins by ATP-dependent cytosolic proteases.

The stress proteins useful in the present invention can be obtained from enterobacteria (*e.g.*, *E. coli*), mycobacteria (particularly *M. leprae*, *M. tuberculosis*, *M. vaccae*, *M. smegmatis*, and *M. bovis*), yeast, *Drosophila*, vertebrates (*e.g.*, avians or mammals such as rodents or primates, including humans).

Protein Expression and Purification

Proteins can be recombinantly produced. More specifically, hsps (or fragments thereof) and HPV antigens (or fragments thereof), which can be administered separately, in combination, or after conjugation, as well as fusion proteins containing component (1) and component (2) can be recombinantly produced in bacteria, yeast, plants or plant cells, or animals or animal cells. For example, hsps, HPV antigens, and fusion proteins containing them can be produced by transformation (*i.e.*, transfection, transduction, or infection) of a host cell with a nucleic acid sequence in a suitable expression vehicle. Suitable expression vehicles include plasmids, viral particles, and phage. For insect cells, baculovirus expression vectors are suitable. The entire expression vehicle, or a part thereof, can be integrated into the host cell genome. In some circumstances, it is desirable to employ an inducible expression vector, for example, the LACSWITCH® Inducible Expression System (Stratagene; La Jolla, CA).

Those skilled in the field of molecular biology will understand that any of a wide variety of expression systems can be used to provide recombinant proteins (*e.g.*, fusion proteins) useful in the methods described herein. The precise host cell and vector used is not critical to the invention.

As noted above, component (1), component (2) and fusion proteins containing them can be produced by plant cells. For plant cells, viral expression vectors (*e.g.*, cauliflower mosaic virus and tobacco mosaic virus) and plasmid expression vectors (*e.g.*, Ti plasmid) are suitable. Such cells are available from a wide range of sources (*e.g.*, the American Type Culture Collection, Manassas, VA; see also, *e.g.*, Ausubel *et al.*, *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, 1994). The methods of transformation and the choice of expression vehicle will depend on the host system selected. Transformation methods are described in, *e.g.*, Ausubel (*supra*). Expression vehicles may be chosen from those provided in, *e.g.*, Pouwels *et al.*, *Cloning Vectors: A Laboratory Manual*, 1985, Supp. 1987.

The host cells harboring the expression vehicle can be cultured in conventional nutrient media adapted as needed for activation or repression of a chosen gene, selection of transformants, or amplification of a chosen gene.

Where appropriate or beneficial, the nucleic acid encoding a fusion protein can include a signal sequence for excretion of the fusion protein to, *e.g.*, facilitate isolation of the protein from a cell culture. Specific initiation signals may also be required for efficient translation of inserted nucleic acid sequences. These signals include the ATG initiation codon and adjacent sequences. In some cases, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of appropriate transcription or translation enhancer elements, (*e.g.*, ones disclosed in Bittner *et al.*, *Methods in Enzymol.* 153:516, 1987).

Component (1), component (2), and fusion proteins containing them can be soluble under normal physiological conditions. In addition, such fusion proteins can include one or more unrelated (*i.e.* a non-hsp, non-HPV) proteins (in whole or in part) to create an, at least, tripartite fusion protein. The "third" protein can be one that facilitates purification, detection, or solubilization of the fusion protein, or that provides some other function. For example, the expression vector pUR278 (Ruther *et al.*, *EMBO J.* 2:1791, 1983) can be used to create lacZ fusion proteins, and the pGEX vectors can be used to express foreign polypeptides as fusion proteins containing glutathione S-transferase (GST). In general, such fusion proteins are soluble and can be easily purified from lysed cells by adsorption to glutathione-agarose beads, followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety. The "third" protein can also be an immunoglobulin Fc domain. Such a fusion protein can be readily purified using an affinity column. Of course, the fusion proteins used in the methods of the invention can include more than one component (1) and/or more than one component (2), and components (1) and (2) may be directly or indirectly linked (*e.g.*, one or more amino acid residues may be present between them).

A protein (*e.g.* an hsp, an HPV antigen or an hsp-containing fusion protein) can be purified by utilizing an antibody to which the protein specifically binds. One of ordinary

skill in the art can use affinity-based purification methods to purify proteins. For example, *see* Janknecht *et al.*, *Proc. Natl. Acad. Sci. USA.* 88:8972, 1981, for purification of non-denatured fusion proteins expressed in human cell lines. In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni^{2+} nitriloacetic acid-agarose columns, and histidine-tagged proteins are selectively eluted with imidazole-containing buffers. The same procedure can be used for a bacterial culture.

Proteins, including fusion proteins (particularly those containing short antigenic fragments), can also be produced by chemical synthesis (*e.g.*, by the methods described in *Solid Phase Peptide Synthesis*, 2nd ed., 1984 The Pierce Chemical Co., Rockford, IL).

Once isolated, the proteins can, if desired, be further purified and/or concentrated, so long as further processing does not impair their ability to elicit an immune response sufficient to be effective in the methods of the invention. A variety of methods for purifying and concentrating proteins are well known in the art (see, *e.g.*, Fisher, *Laboratory Techniques In Biochemistry And Molecular Biology,* Work and Burdon, Eds., Elsevier, 1980), including ultracentrifugation and/or precipitation (*e.g.*, with ammonium sulfate), microfiltration (*e.g.*, via 0.45 μm cellulose acetate filters), ultrafiltration (*e.g.*, with the use of a sizing membrane and recirculation filtration), gel filtration (*e.g.*, columns filled with Sepharose CL-6B, CL-4B, CL-2B, 6B, 4B or 2B, Sephacryl S-400 or S-300, Superose 6 or Ultrogel A2, A4, or A6; all available from Pharmacia Corp.), fast protein liquid chromatography (FPLC), and high performance liquid chromatography (HPLC).

Cross-reactive HPV Sequences

One of ordinary skill in the art can determine whether a composition containing an HPV antigen of a first type can be used to treat a subject who has been infected with a second type of HPV. The assays upon which such a determination can be based include predictive assays (*e.g.*, those employing computer models) and biological assays (in which one actually tests for cross-reactivity). One or both types of assays can be used (not surprisingly, one would expect the results obtained in a predictive assay to be further tested in a biological assay). Examples of each follow.

One can test for cross-reactivity (*i.e.*, the ability of a composition containing an HPV antigen of one type to effectively treat a subject who is infected with an HPV of another type, or who has a disease or condition associated with an HPV of another type) using well-established immunological methods. For example, bi-transgenic mice engineered to express the antigen binding region of the human MHC class I molecule and the human CD8 gene (Lustgarten *et al.*, *Human Immunol.* 52:109, 1997; Vitiello *et al.*, *J. Exp. Med.* 173:1007, 1991) can be used to demonstrate immune cross-reactivity.

More specifically, the HLA-A2/CD8 bi-transgenic mouse (Lustgarten *et al.*, *supra*) can be used to demonstrate cross reactivity of cytotoxic T lymphocytes (CTL) raised to HPV16 E7 against peptides derived from the E7 protein of HPV6 and 11 using standard immunological techniques (*see, e.g.*, Coligan *et al.* Eds., *Current Protocols in Immunology,* John Wiley & Sons, 1999). Briefly, mice are immunized one to three times at intervals of seven to 21 days with HspE7 fusion protein (based on the BCG Hsp65 and HPV16 E7 molecules). HspE7 is suspended in phosphate-buffered saline (PBS) and administered subcutaneously at a dose ranging from 1 μg to 1000 μg per mouse. Seven days following the final administration of HspE7, mice are sacrificed, their spleens removed, and the tissue dissociated into a single cell suspension. CTLs that are specific for HPV E7 are restimulated by the addition of HLA-A2 binding peptides derived from HPV16 E7, HPV6 E7 and HPV11 E7 to the culture medium at a concentration of 1 μM. The cells can be restimulated in, for example, 6-well plates, having a different peptide in each well. The peptides (*e.g.*, the ten peptides) with the highest predicted HLA-A2 binding affinity, as defined by computer algorithm, can be used for each of HPV16, HPV6, and HPV11 (or any other HPV type; see Parker *et al.*, *J. Immunol.* 152:163, 1994; the algorithm is also available on the internet through the BIMAS (Bioinformatics & Molecular Analysis Section) website of the National Institutes of Health (accessed on June 26, 2001 at http://bimas.dcrt.nih.gov/). In addition, where different, the corresponding peptides from the other two HPV genotypes would also be used (*i.e.*, HPV16 E7 peptide 11-20 and HPV6 and 11 peptides 11-20).

Following a period (*e.g.*, one week) of restimulation *in vitro*, CTL activity would be measured by the lysis of T2 target cells pulsed with HLA-A2 binding peptides derived from HPV16 E7, HPV6 E7 and HPV11 E7. In addition, antigen-specific T lymphocytes, which recognize HLA-A2 binding peptides derived from HPV16 E7, HPV6 E7 and HPV11 E7, can

be measured by ELISPOT analysis of IFN-γ secreting cells using previously described methods (Asal *et al. Clin. Diagn. Lab. Immunol.* 7:145, 2000). These analyses could be performed in mice transgenic for other HLA alleles.

Alternately, one can measure the ability of CTL, which are induced by immunization with HspE7, to cross-react with peptides derived from HPV6 or 11 E7 proteins in human HLA-A2 positive subjects undergoing therapy for genital warts using HspE7. Peripheral blood mononuclear cells (PBMC) can be isolated from subjects (*e.g.*, human patients) prior to treatment and several days (*e.g.*, 7 days) following each treatment with HspE7. The cells can be analyzed by fluorogenic MHC-peptide complexes (tetramers, Altman *et al.*, *Science* 274:94, 1996) or by ELISPOT analysis (Asal *et al.*, *Clin. Diagn. Lab. Immunol.* 7:145, 2000). Cells can be assayed directly from the peripheral blood and following *in vitro* restimulation as described by Youde *et al.* (*Cancer Res.* 60:365, 2000). For *in vitro* restimulation, 2 x 10^6/ml PBMC are cultured in RPMI1640 with 10% human AB serum (RAB) and peptide at a concentration of 10 μg/ml. Restimulating peptides would be derived from HPV16 E7 and would comprise the peptides (*e.g.*, the ten peptides) with the highest predicted HLA-A2 binding affinity, as defined by computer algorithm (Parker *et al.*, *supra*). On Day 4, 1 ml of RAB containing 25 units/ml of IL-2 is added to each well. On Day 6, 1 ml of medium is replaced with 1 ml of medium containing 10 units/ml of IL-2. On Day 7, irradiated autologous PBMC (fresh or frozen-then-thawed) are resuspended at 3 x 10^6 cells/ml in RAB containing 10 μg/ml peptide and 3 μg/ml β_2-microglobulin. Antigen presenting cells are allowed to adhere for two hours and are then washed to remove non-adherent cells before the addition of 1-2 x 10^6 effector cells/ml. On Day 9, one ml of RAB containing 25 units/ml of IL-2 is added to each well. On Day 13, the contents of the wells are divided into multiple plates and the medium (containing 10 units/ml of IL-2) is restored to the original volume. The cells are used on Day 14. For FACS analysis, tetramers are prepared as described previously (Altman *et al.*, *Science* 274:94, 1996). The peptides used for loading the tetramers are HLA-A2 binding peptides derived from the E7 molecule of HPV16, HPV6 and HPV11. The peptides (*e.g.*, the ten peptides) with the highest predicted HLA-A2 binding affinity, as defined by computer algorithm (Parker *et al.*, *supra*) are used for each of HPV16, HPV6 and HPV11. In addition, where different, the corresponding peptides from the other two HPV genotypes are also used (*i.e.*, HPV16 E7 peptide 11-20 and

HPV 6 and 11 peptides 11-20). Fresh or restimulated PBMCs are stained with PE-labeled HPV-E7peptide tetramers and FITC labeled anti-CD8 antibody and analyzed by flow cytometry, as has been described. ELISPOT analysis of antigen-specific T lymphocytes that recognize HLA-A2 binding peptides derived from HPV16 E7, HPV6 E7 and HPV11 E7 present in fresh and restimulated PBMC is performed using previously described methods (Asal *et al.*, *Clin. Diagn. Lab. Immunol.* 7:145, 2000). Likewise, these techniques can be applied to subjects with other HLA haplotypes.

In addition, it is possible to test the ability of human PBMC derived from HLA-A2 positive healthy volunteers not previously treated with HspE7, stimulated *in vitro* with HspE7 protein or peptides derived from HPV type 16 E7, to cross-react with cells pulsed with the corresponding peptides from the other two HPV genotypes (6 and 11). Cells are stimulated and assayed using procedures common to the art. Briefly, PBMC are isolated from peripheral blood, adherent cells are separated from non-adherent cells, and the adherent cells are cultured to generate dendritic cells (DC) as described in *Current Protocols in Immunology* (Coligan *et al.*, Eds., John Wiley & Sons, pp 7.32.7-8, 1999). The non-adherent cells are cryopreserved in 90% FCS/10% DMSO for use at a later point in the assay.

For the stimulation, DC are pulsed with 50 μg/ml HspE7 or with 40 μg/ml of the appropriate peptide and 3 μg/ml β_2-microglobulin for 24 hours at 37°C, 5% CO_2 (Kawashima *et al.*, *Human Immunol.* 59:1, 1998). The peptides used are HLA-A2 binding peptides derived from the E7 molecule of HPV16, HPV6 and HPV11. The peptides (*e.g.*, the ten peptides) with the highest predicted HLA-A2 binding affinity, as defined by computer algorithm (Parker *et al.*, *supra*) are used for each of HPV16, HPV6 and HPV11. In addition, where different, the corresponding peptides from the other two HPV genotypes would also be used (ie HPV16 E7 peptide 11-20 and HPV 6 and 11 peptides 11-20). $CD8^+$ cells are isolated from cryopreserved, autologous non-adherent cells by positive selection using immunomagnetic beads (Miltenyi Biotec). Peptide/protein-loaded DC are irradiated at 4200 rads and mixed with autologous $CD8^+$ cells at a ratio of 1:20 in, *e.g.*, 48-well plates containing 0.25 x 10^5 DC and 5 x 10^5 $CD8^+$ cells and 10 ng/ml of IL-7 in 0.5 mls of RAB. On days 7 and 14, the cells are restimulated with autologous peptide-pulsed adherent APC (Kawashima *et al.*, *Human Immunol.* 59:1, 1998). The cultures are fed every 2-3 days with 10 U/ml of hIL-2. HPV E7 peptide-specific T lymphocytes are analyzed by fluorogenic

MHC-peptide complexes (tetramers, Altman *et al.*, *Science* 274:94, 1996) or by ELISPOT analysis (Asal *et al.*, *Clin. Diagn. Lab. Immunol.* 7:145, 2000) following 7 and 14 days of *in vitro* stimulation. For FACS analysis, tetramers are prepared as described previously (Altman *et al. Science* 274:94, 1996). The peptides used for loading the tetramers would be HLA-A2 binding peptides derived from the E7 molecule of HPV16, HPV6 and HPV11, as described above. Peptide specific T lymphocytes are stained with PE-labeled HPV-E7 peptide tetramers and FITC labeled anti-CD8 antibody and analyzed by flow cytometry (Youde *et al. Cancer Res.* 60:365, 2000). ELISPOT analysis of antigen-specific T lymphocytes, which recognize HLA-A2 binding peptides derived from HPV16 E7, HPV6 E7 and HPV11 E7, is performed using previously described methods (Asal *et al. Clin. Diagn. Lab. Immunol.* 7:145, 2000). Likewise, these techniques could be applied to subjects with other HLA haplotypes.

Administration of Compositions

The invention includes compositions containing at least one HPV protein antigen (*e.g.* an HPV protein antigen (or an antigenic fragment thereof), an HPV protein antigen mixed with or conjugated to an hsp (or an immunostimulatory fragment thereof) or a fusion protein containing an HPV protein antigen (or an antigenic fragment thereof) and an hsp (or an immunostimulatory fragment thereof). Optionally, these proteins can be suspended in a pharmaceutically acceptable carrier, such as a diluent (*e.g.*, PBS) or a bicarbonate solution (*e.g.*, 0.24 M $NaHCO_3$). Useful carriers are selected on the basis of the mode and route of administration and on standard pharmaceutical practice. Suitable pharmaceutical carriers and diluents, as well as pharmaceutical necessities for their use, are described in Remington's Pharmaceutical Sciences. An adjuvant, for example, a cholera toxin, *Escherichia coli* heat-labile enterotoxin (LT), a liposome, or an immune-stimulating complex (ISCOM), can also be included.

The protein(s) (*e.g.*, the fusion protein) need not be administered to the subject directly. Instead, a nucleic acid sequence encoding the protein can be administered; the protein being expressed in the subject *in vivo*. The nucleic acid can be a part of a vector (such as a viral vector, for example, a part of a viral vector genome), or encapsulated, for

example, in liposomes. Alternatively, the nucleic acid can be delivered as a naked nucleic acid.

The compositions can be formulated as a solution, suspension, suppository, tablet, granules, a powder, a capsule, ointment, or cream. As noted above, in preparing these compositions, one or more pharmaceutical carriers can be included. Additional examples of pharmaceutically acceptable carriers or other additives include solvents (*e.g.*, water or physiological saline), solubilizing agents (*e.g.*, ethanol, polysorbates, or Cremophor EL®), agents for rendering isotonicity, preservatives, antioxidizing agents, excipients (*e.g.*, lactose, starch, crystalline cellulose, mannitol, maltose, calcium hydrogen phosphate, light silicic acid anhydride, or calcium carbonate), binders (*e.g.*, starch, polyvinylpyrrolidone, hydroxypropyl cellulose, ethyl cellulose, carboxy methyl cellulose, or gum arabic), lubricants (*e.g.*, magnesium stearate, talc, or hardened oils), or stabilizers (*e.g.*, lactose, mannitol, maltose, polysorbates, macrogels, or polyoxyethylene-hardened castor oils). If necessary (or desired), glycerin, dimethylacetamide, sodium lactate, a surfactant, sodium hydroxide, ethylenediamine, ethanolamine, sodium bicarbonate, arginine, meglumine, or trisaminomethane can be added. Biodegradable polymers such as poly-D,L-lactide-co-glycolide or polyglycolide can be used as a bulk matrix if slow release of the composition is desired (see, for example, U.S. Patent Nos. 5,417,986, 4,675,381, and 4,450,150). Pharmaceutical preparations such as solutions, tablets, granules or capsules can be formed with these components. If the composition is administered orally, flavorings and colors can be added.

The therapeutic compositions can be administered via any appropriate route, for example, intravenously, intraarterially, topically, intraperitoneally, intrapleurally, orally, subcutaneously, intramuscularly, intradermally, sublingually, intraepidermally, nasally, intrapulmonarily (*e.g.*, by inhalation), vaginally, or rectally.

The amount of the composition administered will depend, for example, on the particular composition, whether an adjuvant is co-administered with the composition, the type of adjuvant co-administered, the mode and frequency of administration, and the desired effect (*e.g.*, protection or treatment). Dosages are routinely determined by those of ordinary skill in the art in the course of developing drugs or prophylactic agents. In general, the compositions of the present invention are administered in amounts ranging between 1 μg and

100 mg per adult human dose. If adjuvants are administered with the compositions, amounts ranging between 1 ng and 1 mg per adult human dose can generally be used. Administration is repeated as necessary, as can be determined by one of ordinary skill in the art. For example, a priming dose can be followed by three booster doses at weekly or monthly intervals. A booster shot can be given at 3 to 12 weeks after the first administration, and a second booster can be given 3 to 12 weeks later, using the same formulation. Serum or T cells can be taken from the subject for testing the immune response elicited by the composition against the HPV antigen included in, for example, the fusion protein or protein conjugate. Methods of assaying antibodies or cytotoxic T cells against a specific antigen are well known in the art. Additional boosters can be given as needed. By varying the amount of, for example, fusion protein in the composition, the immunization protocol can be optimized for eliciting a maximal immune response.

Of course, the proteins described herein can also be delivered by administering a nucleic acid, such as a viral vector (e.g., a retroviral or adenoviral vector).

Without further elaboration, it is believed that one skilled in the art can, based on the above disclosure and the example below, utilize the present invention to its fullest extent. The following example is to be construed as merely illustrative of how one skilled in the art can isolate and use fusion polypeptides, and is not limitative of the remainder of the disclosure in any way. All publications, including U.S. patents and published International applications, cited herein are hereby incorporated by reference

EXAMPLE

A fusion polypeptide containing the *M. bovis* BCG Hsp65 coupled to the E7 protein of HPV type 16 was recombinantly produced and formulated as described in WO 99/07860. Hsp65 is a member of the Hsp60 family of stress proteins. In the course of a human clinical trial for testing the efficacy of this fusion polypeptide in the treatment of anal high-grade squamous intraepithelial lesions (HSIL), the following observation was made.

Twenty-two patients participated in a randomized, double-blind, placebo-controlled, multicenter trial of HspE7 in the treatment of anal HSIL. Eligible patients had biopsy-confirmed anal HSIL and were negative for human immunodeficiency virus (HIV). Patients were typed for HPV using cells obtained from an anal swab, but were not required to have

HPV-16. Individual lesions were not typed for HPV. Patients received three subcutaneous injections of either 100 μg of HpsE7 or placebo at monthly intervals. They were assessed for treatment response by anal Pap smears, high-resolution anoscopy (HRA) with biopsy, and global physician assessment. Non-responders (*i.e.*, those with persistent anal HSIL) after 12 or 24 weeks in the controlled trial were allowed to crossover to an open-label trial where they received three injections of 500 μg of HspE7 at monthly intervals. The treatment assignment was double-blinded in the placebo-controlled trial, and the blind has not been broken.

To determine the HPV type(s) infecting patients, a Dacron swab was used to collect specimens from the anus of patients at the screening visit of the randomized, placebo-controlled trial, just before biopsy. After transport in Sample Transport Medium (Digene), DNA was isolated and used to determine HPV type. Briefly, the consensus primer set MY09/MY11 was used to amplify HPV DNA by polymerase chain reaction (PCR). Following the amplification step, samples were blotted onto nylon membrane and probed with biotin-labeled oligonucleotides specific for 29 different HPV types (6, 11, 16, 18, 26, 31, 32, 33, 35, 39, 40, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 66, 68, 69, 70, 73, AE2, Pap155, and Pap291), plus a pooled probe containing primers for 10 HPV types (2, 13, 34, 42, 57, 62, 64, 67, 72, and W13B). Samples that produced a "dot blot" were scored positive or negative for HPV type by comparison to standardized controls using a 5-point scale; a score of 1 or greater was positive.

To verify that the PCR was successful, a beta-globin control amplification and probe detection was performed for each sample. If the sample was not positive for the presence of beta-globin, the PCR step was considered a technical failure. If the consensus probe did not result in a score of 2 or more, the sample was considered "HPV negative."

At the time of their entry into the open label trial, 14 of the 22 patients (64%) had anogenital warts that had persisted throughout the prior double-blind trial in which they received three monthly injections of either 100 μg of HspE7 or placebo. Of these 14 patients, 8 patients (57%) had worsened, 4 patients (29%) had no change, and 2 patients (14%) improved (one dramatically and the other minimally) by the time they crossed over to the open label trial. An additional patient had condyloma present at the start of the double-blind trial that resolved before the start of the open-label trial, and is omitted from this analysis, as are the seven other patients who had no detectable warts during either trial.

Condylomata were present within the anorectal canal in all 14 patients (100%) and on the perianal skin as well in 6 of 14 patients (43%). Of the 14 patients with warts at the beginning of the open label trial, the site investigator determined that surgical ablation was needed for 11 (79%) patients, local ablation (*e.g.*, liquid nitrogen, electrocautery) was needed for 2 patients (14%), and topical treatment (*i.e.*, imiquimod) was needed for 1 patient (7%). These patients elected to postpone the site investigator's recommended treatment, consenting instead to receive three injections of HspE7 500 µg at monthly intervals in the open label trial.

One month after the final treatment with 500 µg of HspE7, 2 patients (14%) had no detectable warts, 11 patients (79%) had a reduction in the size or number of warts as compared with their status upon entry into the open-label trial, and 1 patient (7%) experienced an increase in wart size (Table 1). By the time of the primary evaluation point of the open label trial (4 months after the final dose) one additional patient experienced an improvement from partial to complete response (*i.e.*, no visible warts), giving a total of three (21%) complete responders (Table 1). None of these responders relapsed during the six months of evaluation in the open label trial. Ten patients (71%) continued to exhibit improvement in partial response (*i.e.*, warts reduced further in size significantly with continued diminution of the extent of treatment needed to remove the remaining warts). The one non-responder (7%) did not improve by the end of the open-label trial.

TABLE 1

Response Summary for Anogenital Warts After Treatment with HspE7

	Number (%) of Patients	
Outcome	Week 12* (n=14)	Week 24† (n=14)
Complete responder	2 (14)	3 (21)
Partial responder	11 (79)	10 (71)
Non-responder	1 (7)	1 (7)

* One month after the final treatment with 500 µg of HspE7.

† Four months after the final treatment with 500 µg of HspE7.

At the end of the trial, the site investigator did not recommend further treatment for the three complete responders. As listed in Table 2, the site investigator's recommended treatment for the partial responders was ablative therapy (6 of 14, 43%) or treatment with a topical agent (4 of 14, 29%); additional surgery was recommended for the non-responder (1 of 14, 7%). All 22 patients entered a registry protocol for long-term follow-up of their response and they consented to postpone the investigator's recommended treatment.

TABLE 2

Anogenital Wart Response Assessments and Clinician-Recommended Treatment

Patient Number	Baseline*	Week 12†		Week 24‡	
	Recommended Treatment	Wart Response	Recommended Treatment	Wart Response	Recommended Treatment
003	Surgery	CR	Topical	CR	None
004	Surgery	PR	Ablation	PR	Ablation
005	Topical	PR	Topical	CR	None
006	Surgery	PR	Surgery	PR	Ablation
008	Ablation	PR	Topical	PR	Topical
009	Surgery	PR	Ablation	PR	Topical
010	Surgery	PR	Ablation	PR	Ablation
011	Surgery	CR	Topical	CR	None
014	Surgery	PR	Topical	PR	Topical
016	Surgery	Worse	Surgery	Worse	Surgery
017	Ablation	PR	Topical	PR	Topical

TABLE 2

Anogenital Wart Response Assessments and Clinician-Recommended Treatment

Patient Number	Baseline*	Week 12†		Week 24‡	
	Recommended Treatment	Wart Response	Recommended Treatment	Wart Response	Recommended Treatment
020	Surgery	PR	Ablation	PR	Ablation
021	Surgery	PR	Ablation	PR	Ablation
022	Surgery	PR	Ablation	PR	Ablation

* Baseline refers to the beginning of the open-label trial.

† One month after the final treatment with 500 μg of HspE7.

‡ Four months after the final treatment with 500 μg of HspE7.

Abbreviations: CR = complete response; PR = partial response

In all 14 patients diagnosed with anogenital warts, HPV DNA of multiple HPV types was detected in anal swab specimens during screening for the first, randomized, controlled trial (Table 3). HPV-6 and/or 11 were present in 12 patients (86%). One patient had only HPV-16 and related types and another patient could not be typed. Three of the 14 patients (21%) were positive for HPV-16. Most patients whose warts improved (11 of 13, 85%) did not have HPV-16. The non-responder also did not have HPV-16 (see Table 3).

TABLE 3

HPV Types in Patients with Anogenital Warts

Patient Number	HPV Type in Anal Swab Specimens, at Screen*	Wart Response at Week 24†
003	6, 11, 16	CR
004	6, 54	PR
005	6, 70	CR
006	6, 11, 45	PR

TABLE 3

HPV Types in Patients with Anogenital Warts

Patient Number	HPV Type in Anal Swab Specimens, at Screen*	Wart Response at Week 24†
008	16, 31, 55	PR
009	6, 11, 59	PR
010	6, 11, 45, 54	PR
011	HPV positive, type unknown	CR
014	6, 11	PR
016	11, 61	Worse
017	HPV negative	PR
020	6, 11, 16	PR
021	6, 31, 53, 58, 59, 61, 66	PR
022	6	PR

* Screening visit of the randomized, placebo-controlled clinical trial.

† Four months after the final treatment with 500 μg of HspE7.

Abbreviations: HPV = human papillomavirus; CR = complete response; PR = partial response

In this open-label, crossover trial of HspE7 (500 μg at 3 monthly intervals) involving patients with persistent anal HSIL and concomitant anogenital warts, 3 of the 14 patients (17%) who had warts at baseline no longer had warts 4 months after the final dose. Another 10 patients (71%) experienced improvement in their symptoms (*i.e.*, warts reduced in size significantly and continued diminution of the extent of treatment needed to remove the remaining warts). One patient (7%) did not improve over the course of the trial and additional surgery was recommended by the site investigator.

Before enrollment in the open-label trial, most patients at this trial site would have undergone surgical intervention for the removal of their warts (11 of 14, 79%). By the end of the trial, surgical treatment was recommended for only one patient. Local ablative therapy (*e.g.*, liquid nitrogen, electrocautery) was recommended for six patients (43%) and treatment

with a topical agent (*e.g.*, imiquimod) was recommended for four patients (29%). Three patients did not need further treatment.

Responses appear to be progressive over 6 months and no responder relapsed over this period. Gradual and progressive resolution of condyloma is in keeping with what one would expect from an immunologic host response after induction of cell-mediated immunity by HspE7.

Two patients in the double-blind trial had some improvement in their condyloma before entering the open-label trial. To date, we have not broken the blind and do not know whether these patients received 100 μg of HspE7 or placebo. However, based on the response observed in the open-label trial of three monthly injections of 500 μg of HspE7, it appears that the higher dose is more active than 100 μg.

HPV-16 DNA was detected in anal swab specimens from only 3 of the 13 patients (23%) whose warts improved after treatment with HspE7. DNA from HPV-6, HPV-11, or both, was detected in most of the patients whose warts responded to treatment with HspE7. These data suggest that there is immunologic cross-reactivity between these HPV types in their response to HspE7.

In summary, the results presented here suggest that HspE7 is broadly active in anogenital warts. This activity does not appear to be limited to HPV-16 positive patients, but crosses multiple HPV types. It is predicted that HspE7 will be active in the treatment of HPV-induced diseases of the anogenital region and that this activity will not be limited to HPV-16 positive patients.

The observations reported here suggest that therapeutic treatment with HspE7 may constitute a new, simple, and non-surgical treatment for anogenital warts that, at a minimum, would lessen wart burden, thereby reducing the extent of treatment and resultant morbidity. Internal anorectal disease often requires additional treatment that can be quite painful and debilitating. Any treatment that provides a even partial response that reduces or eliminates the amount or extent of "surgical" or ablative therapy translates into a reduction in morbidity, less loss of time from work, and improved quality of life.

These results indicate that a heat shock protein/HPV type 16 antigen composition is effective in eliminating or reducing warts, which are thought to be caused predominantly by HPV types 6 and 11. Of significant import are the observations that (1) warts can be treated

at all with an HPV-based composition, and (2) a HPV type 16 composition was effective in treating a condition presumably caused by a HPV other than type 16. The latter cross-reactive result was wholly unexpected, given the generally held belief that a type-specific composition could only elicit a type-specific immune response.

To elucidate a possible mechanism for the observed cross-reactivity of the fusion polypeptide, theoretic binding was calculated for various HLA class I molecules and E7 peptides of HPV types 16, 6, and 11. The $T_{1/2}$ of dissociation was calculated using the algorithm described in Parker *et al.*, *J. Immunol.* 152:163, 1994 (see also the website described above). The data is summarized in Table 4.

TABLE 4

HLA Type	HPV Type 16			HPV Type 6			HPV Type 11		
	start	sequence	$T_{1/2}$	start	sequence	$T_{1/2}$	start	sequence	$T_{1/2}$
A1	**44**	**QAEPDRAHY**	**900**	**44**	**DSQPLKQHY**	**8**	44	DAQPLTQHY	5
	16	**QPETTDLYCY**	**23**	**17**	**PPDPVGLHCY**	**6**	**17**	**PPDPVGLHCY**	**6**
							68	**VVECTDGDIR**	**9**
A_0201	**11**	**YMLDLQPET**	**375**						
	7	**TLHEYMLDL**	**201**	7	TLKDIVLDL	7	7	TLKDIVLDL	7
	82	LLMGTLGIV	54	**82**	**LLLGTLNIV**	**412**	**82**	**LLLGTLNIV**	**412**
				28	**QLVDSSEDEV**	**140**	28	QLEDSSEDEV	9
	78	TLEDLLMGT	5	79	VQQLLLGTL	1	**78**	**QLQDLLLGT**	**70**
A_0205	**7**	**TLHEYMLDL**	**50**	7	TLKDIVLDL	4	7	TLKDIVLDL	4
	11	**YMLDLQPET**	**27**	12	VLDLQPPDPV	1	12	VLDLQPPDPV	1
	82	LLMGTLGIV	20	**82**	**LLLGTLNIV**	**20**	82	LLLGTLNIV	20
	78	TLEDLLMGT	2	**79**	**VQQLLLGTL**	**19**	**78**	**QLQDLLLGT**	**42**
	5	TPTLHEYML	0	5	HVTLKDIVL	14	**5**	**LVTLKDIVL**	**24**
A24	**56**	**TFCCKCDSTL**	**20**						
	51	**HYNIVTFCC**	**11**	**51**	**HYQIVTCCC**	**11**	51	HYQILTCCC	9
	24	CYEQLNDSS	9	**25**	**CYEQLVDSS**	**9**	25	CYEQLEDSS	9
	4	DTPTLHEYML	6	5	HVTLKDIVL	4	**4**	**RLVTLKDIVL**	**12**
				39	EVDGQDSQPL	5	**39**	**KVDKQDAQPL**	**10**
A3	**88**	**GIVCPICSQK**	**14**	**88**	**NIVCPICAPK**	**5**	**88**	**NIVCPICAPK**	**5**
	7	**TLHEYMLDL**	**8**	**7**	**TLKDIVLDL**	**5**	**7**	**TLKDIVLDL**	**5**
A68.1	**68**	**CVQSTHVDIR**	**200**	**68**	**VVQCTETDIR**	**200**	**68**	**VVECTDGDIR**	**200**
	89	**IVCPICSQK**	**180**	**89**	**IVCPICAPK**	**180**	**89**	**IVCPICAPK**	**180**
A_1101	**89**	**IVCPICSQK**	**2.0**	**89**	**IVCPICAPK**	**2**	**89**	**IVCPICAPK**	**2.0**
	68	**CVQSTHVDIR**	**1.8**	**68**	**VVQCTETDIR**	**1**	**68**	**VVQCTETDIR**	**0.6**
A_3101	**69**	**VQSTHVDIR**	**4.0**	**69**	**VQCTETDIR**	**2**	**68**	**VVECTDGDIR**	**2.0**

HLA Type	HPV Type 16			HPV Type 6			HPV Type 11		
	start	sequence	$T_{1/2}$	start	sequenc	$T_{1/2}$	start	s quence	$T_{1/2}$
	88	**GIVCPICSQK**	**0.4**	**88**	**NIVCPICAPK**	**0**	**88**	**NIVCPICAPK**	**0.4**
A_3302	**68**	**CVQSTHVDIR**	**15**	**68**	**VVQCTETDIR**	**15**	**68**	**VVECTDGDIR**	**15**
	58	**CCKCDSTLR**	**3**	**58**	**CCGCDSNVR**	**3**	**58**	**CCGCDSNVR**	**3**
B14	**65**	**LRLCVQSTHV**	**30**	65	VRLVVQCTE	1	65	VRLVVECTD	1
	4	**DTPTLHEYML**	**18**	**3**	**GRHVTLKDI**	**12**	**3**	**GRLVTLKDI**	**60**
	5	TPTLHEYML	3	**6**	**VTLKDIVLDL**	**10**	6	VTLKDIVLDL	15
	76	RTLEDLLMGT	1	76	IREVQQLLL	4	**76**	**IRQLQDLLL**	**20**
B40	**36**	**DEIDGPAGQA**	**120**	35	DEVDEVDGQ	2	35	DEVDKVDKQ	2
	74	**VDIRTLEDL**	**10**	**74**	**TDIREVQQL**	**10**	**74**	**GDIRQLQDL**	**20**
	77	RTLEDLLMGT	0	**77**	**REVQQLLLGT**	**16**			
	87	LGIVCPICS	0	87	LNIVCPICA	2	**87**	**LNIVCPICA**	**2**
B60	**79**	**LEDLLMGTL**	**176**	79	VQQLLLGTL	2	79	LQDLLLGTL	2
	20	**TDLYCYEQL**	**44**	21	VGLHCYEQL	9	21	VGLHCYEQL	9
	74	VDIRTLEDL	40	**74**	**TDIREVQQL**	**44**	**74**	**GDIRQVQDL**	**44**
				40	**VDGQDSQPL**	**20**	**40**	**VDKQDAQPL**	**20**
B61	**36**	**DEIDGPAGQA**	**40**	35	DEVDEVDG	1	35	DEVDKVDKQ	1
	34	**EEDEIDGPA**	**20**	**33**	**SEDEVDEV**	**40**	**33**	**SEDEVDKV**	**40**
				72	**TETDIREV**	**80**	72	TDGDIRQL	1
	29	NDSSEEEDEI	1				**29**	**LEDSSEDEV**	**40**
B62	**15**	**LQPETTDLY**	**88**	15	LQPPDPVGL	6	15	LQPPDPVGL	6
	43	**GQAEPDRAHY**	**44**	44	DSQPLKQHY	1	44	DAQPLTQHY	5
	7	TLHEYMLDL	3	**7**	**TLKDIVLDL**	**16**	**7**	**TLKDIVLDL**	**16**
	82	LLMGTLGIV	2	**83**	**LLGTLNIVC**	**11**	**83**	**LLGTLNIVC**	**11**
B7	**5**	**TPTLHEYML**	**80**	**5**	**HVTLKDIVL**	**20**	5	LVTLKDIVL	20
	75	**DIRTLEDLL**	**40**	**75**	**DIREVQQLL**	**40**	**75**	**DIRQLQDLL**	**40**
	46	EPDRAHYNI	2	46	QPLKQHYQI	8	**46**	**QPLTQHYQIL**	**80**
B8	**58**	**CCKCDSTLRL**	**16**	58	CCGCDSNVRL	1	58	CCGCDSNVRL	1
	75	**DIRTLEDL**	**8**	**75**	**DIREVQQL**	**12**	**75**	**DIRQLQDLL**	**8**
				7	**TLKDIVLDL**	**12**	**7**	**TLKDIVLDL**	**12**
B_2702	**48**	**DRAHYNIVTF**	**60**	49	KQHYQIVTC	6	49	TQHYQILTC	2
	76	**IRTLEDLLM**	**20**	**76**	**IREVQQLLL**	**60**	**76**	**IRQLQDLLL**	**60**
	65	LRLCVQSTH	20	**65**	**VRLVVQCTET**	**20**	65	VRLVVECTD	2
	2	HGDTPTLHEY	1	3	GRHVTLKDIV	20	**3**	**GRLVTLKDIV**	**20**
B_2705	**76**	**IRTLEDLLM**	**600**	**76**	**IREVQQLLL**	**2000**	**76**	**IRQLQDLLL**	**2000**
	65	**LRLCVQSTHV**	**600**	65	VRLVVQCTET	200	65	VRLVVECTD	20
				3	**GRHVTLKDIV**	**600**	3	**GRLVTLKDIV**	**600**
B_3501	**5**	**TPTLHEYML**	**20**	5	HVTLKDIVL	1	5	LVTLKDIVL	1
	16	**QPETTDLYCY**	**18**	15	LQPPDPVGL	2	15	LQPPDPVGL	2
	43	GQAEPDRAHY	6	**44**	**DSQPLKQHY**	**10**	**44**	**DAQPLTQHY**	**6**
	46	EPDRAHYNIV	1	**46**	**QPLKQHYQI**	**8**	**46**	**QPLTQHYQIL**	**20**
B_3701	**74**	**VDIRTLEDLL**	**200**	**74**	**TDIREVQQLL**	**300**	**74**	**GDIRQLQDLL**	**200**

HLA Type	HPV Type 16			HPV Type 6			HPV Type 11		
	start	sequ nc	$T_{1/2}$	start	sequenc	$T_{1/2}$	start	s qu nc	$T_{1/2}$
	20	**TDLYCYEQL**	**40**	21	VGLHCYEQLV	1	21	VGLHCYEQL	1
				40	**VDGQDSQPL**	**40**	40	VDKQDAQPL	40
	80	EDLLMGTLGI	40	80	QQLLLGTLNI	1	**80**	**QDLLLGTLNI**	**40**
B_3801	**78**	**TLEDLLLMGTL**	**8**	78	EVQQLLLGTL	1	**79**	**LQDLLLGTL**	**4**
	50	**AHYNIVTFC**	**4**	50	QHYQIVTCC	4	50	QHYQILTCC	4
	5	TPTLHEYML	2	**4**	**RHVTLKDIVL**	**30**	4	RLVTLKDIVL	1
				39	**EVDGQDSQPL**	**6**	39	KVDKQDAQPL	3
	71	STHVDIRTL	1	71	CTETDIREV	1	**71**	**CTDGDIRQL**	**6**
B_3901	**78**	**TLEDLLMGTL**	**27**	79	VQQLLLGTL	5	**79**	**LQDLLLGTL**	**14**
	73	**HVDIRTLEDL**	**14**	73	ETDIREVQQL	14	74	GDIRQLQDL	1
	77	RTLEDLLMGT	1	**76**	**IREVQQLLL**	**45**	75	DIRQLQDLL	1
	4	DTPTLHEYML	2	**4**	**RHVTLKDIVL**	**90**	**3**	**GRLVTLKDI**	**15**
B_3902	**59**	**CKCDSTLRL**	**20**	59	CGCDSNVRL	2	59	CGCDSNVRLV	3
	7	**TLHEYMLDL**	**2**	7	TLKDIVLDL	1	6	VTLKDIVLDL	9
	79	LEDLLMGTL	1	**79**	**VQQLLLGTL**	**24**	**79**	**LQLQDLLLGTL**	**24**
	15	LQPETTDLY	1	**15**	**LQPPDVGL**	**20**	**15**	**LQPPDPVGL**	**20**
B_4403	**36**	**DEIDGPAGQA**	**90**	35	DEVDEVDGQ	7	**35**	**DEVDKVDKQ**	**16**
	3	**GDTPTLHEY**	**45**						
	44	QAEPDRAHY	6	**44**	**DSQPLKQHY**	**18**	**44**	**DAQPLTQHY**	**27**
				77	**REVQQLLLGT**	**12**			
B_5101	**46**	**EPDRAHYNI**	**880**	**46**	**QPLKQHYQI**	**440**	**46**	**QPLTQHYQI**	**400**
	84	**MGTLGIVCPI**	**114**	**84**	**LGTLNIVCPI**	**114**	**84**	**LGTLNIVCPI**	**114**
B_5102	**46**	**EPDRAHYNI**	**220**	**46**	**QPLKQHYQI**	**1452**	**46**	**QPLTQHYQI**	**1452**
	84	**MGTLGIVCPI**	**88**	84	LGTLNIVCPI	88	**84**	**LGTLNIVCPI**	**88**
				21	**VGLHCYEQLV**	**145**	21	VGLHCYEQL	73
B_5103	**46**	**EPDRAHYNI**	**58**	**46**	**QPLKQHYQI**	**83**	**46**	**QPLTQHYQI**	**58**
	84	**MGTLGIVCPI**	**44**	84	LGTLNIVCPI	44	**84**	**LGTLNIVCPI**	**44**
				21	**VGLHCYEQLV**	**53**			
B_5201	**46**	**EPDRAHYNIV**	**100**	**46**	**QPLKQHYQIV**	**132**	46	QPLTQHYQIL	22
	81	**DLLMGTLGIV**	**33**	**82**	**LLLGTLNIV**	**50**	**82**	**LLLGTLNIV**	**50**
				60	GCDSNVRLVV	40	**60**	**GCDSNVRLVV**	**40**
B_5801	**49**	**RAHYNIVTF**	**79.0**	49	KQHYQIVTCC	0	48.0	LTQHYQILTC	3.0
	77	**RTLEDLLMGT**	**24.0**	77	REVQQLLLGT	0	77.0	RQLQDLLLGT	0.1
	6	PTLHEYMLDL	0.2	**6**	**VTLKDIVLDL**	**8**	**6.0**	**VTLKDIVLDL**	**8.0**
	44	QAEPDRAHY	6.0	**44**	**DSQPLKQHY**	**5**	44.0	DAQPLTQHY	3.0
	85	GTLGIVCPI	4.0	85	GTLNIVCPI	4	**85.0**	**GTLNIVCPI**	**4.0**
Cw_0301	**20**	**TDLYCYEQL**	**100**	**21**	**VGLHCYEQL**	**100**	**21**	**VGLHCYEQL**	**100**
	74	**VDIRTLEDL**	**30**	**74**	**TDIREVQQL**	**36**	74	GDIRQLQDL	36
				46	QPLKQHYQIV	6	**46**	**QPLTQHYQIL**	**120**
Cw_0401	**56**	**TFCCKCDSTL**	**200**				57	CCCGCDSNV	1
	5	**TPTLHEYML**	**88**						
	73	HVDIRTLEDL	14	**73**	**ETDIREVQQL**	**12**	**73**	**DGDIRQLQDL**	**12**

HLA Type	HPV Typ 16			HPV Type 6			HPV Type 11		
	start	s qu nc	$T_{1/2}$	start	sequence	$T_{1/2}$	start	sequenc	$T_{1/2}$
	46	EPDRAHYNI	17	**46**	**QPLKQHYQIV**	**11**	**46**	**QPLTQHYQIL**	**88**
Cw_0602	**79**	**LEDLLMGTL**	**6**	**79**	**VQQLLLGTL**	**13**	**79**	**LQDLLLGTL**	**13**
	85	**GTLGIVCPI**	**6**	85	GTLNIVCPI	6	85	GTLNIVCPI	6
	7	TLHEYMLDL	2	**7**	**TLKDIVLDL**	**12**	**7**	**TLKDIVLDL**	**12**
Cw_0702	**3**	**GDTPTLHEY**	**27**	3	GRHVTLKDI	1	3	GRLVTLKDI	1
	15	**LQPETTDLY**	**8**	16	QPPDPVGLHC	3	16	QPPDPVGLHC	3
	43	GQAEPDRAHY	2	**43**	**QDSQPLKQHY**	**11**	**43**	**QDAQPLTQHY**	**32**
	7	TLHEYMLDL	1	**7**	**TLKDIVLDL**	**4**	**7**	**TLKDIVLDL**	**4**

The peptide sequences in bold indicate the top two binders for each HLA molecule, and for each the E7 protein from each HPV type.

The results in Table 4 suggest that, depending on the specific HLA molecule examined, the HPV type 16 E7 antigen may trigger a cell mediated immune response against the E7 antigen of other HPV types. For example, for HLA B 2705, a high level of binding was predicted for peptides starting from amino acid position 76 of E7 for all three HPV types. Thus, it is possible that, for patients expressing this HLA molecule, an HPV type 16 E7 composition would be cross-reactive and useful for treating or preventing infection by HPV types 6 and 11. Each of the bolded peptide fragments in Table 4 represents a possible antigenic fragment that can be included in the compositions (*e.g.*, the fusion polypeptides described herein), as a substitute for the complete E7 viral antigen. Of course, two or more such putative HLA epitopes, or a long fragment containing many putative HLA epitopes, can also be used.

What is claimed is: